BIOGRAPHICAL SKETCH

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NAME: Robert F. Kalejta

eRA COMMONS USER NAME (credential, e.g., agency login): rfkalejta

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Pennsylvania State University, University Park, PA	BS	1990	Biochemistry
University of Virginia, Charlottesville, VA	Ph.D.	1997	Biochemistry
Princeton University, Princeton, NJ	postdoc	1996-2003	Virology

A. Personal Statement

I have studied human cytomegalovirus (HCMV) for 20 years, since I was a post-doc at Princeton with Tom Shenk. I am humbled by and grateful for my extramural funding, which has been continuous since January 1st 2004, four months after starting as an Assistant Professor at the University of Wisconsin-Madison. I have trained or am currently training 10 postdocs, 17 graduate students, and 8 undergraduates. I served on the VIRB NIH study section, am on the Editorial Board of the Journal of Virology, am an Associate Editor of PLoS Pathogens, and regularly attend ASM, ASV, GRC, IHW and Keystone virology conferences, all of which keep me in tune with current and cutting-edge virology research. The two major research directions of my lab are how cell cycle pathways promote and are modulated by HCMV infection, and how viral immediate early (IE) transcription is initiated at the start of a lytic infection but silenced when latency is established.

B. Positions and Honors

2003-2009 Assistant Professor of Oncology and Molecular Virology, University of Wisconsin-Madison
2009-2014 Associate Professor of Oncology and Molecular Virology, University of Wisconsin-Madison
2011-present Assistant Director, McArdle Laboratory for Cancer Research (IMV Liaison), UW-Madison
2013-present Vice-Chair, Institute for Molecular Virology, UW-Madison
2014-present Professor of Oncology and Molecular Virology, University of Wisconsin-Madison

1993-1996 NIH Cell and Molecular Biology Training Grant T32 GM08136
1997-2000 Leukemia and Lymphoma Society Fellow
2004-2007 American Heart Association Scientist Development Award
2006-2012 Burroughs Wellcome Fund Investigator in Pathogenesis of Infectious Disease
2009 Plenary Speaker, American Society for Virology Conference
2010-2018 Editorial Board, The Journal of Virology
2010-2011 Ad Hoc NIH study section VIRA (Feb 2010; Oct 2010) and VIRB (Feb 2011).
2011-2015 Member, Virology – B study section
2011-2012 Vilas Associate
2012 State of the Art Speaker, American Society for Virology Conference
2012 Keynote Speaker, International Herpesvirus Workshop
2014-2017 Associate Editor, PLoS Pathogens
2016 Organizer, 41st International Herpesvirus Workshop

C. Contributions to Science

1. Discovery of intrinsic defenses against both lytic and latent HCMV

Intrinsic defenses are cell-autonomous components of the immune system that protect individual cells from viral attack. Mediated by constitutively expressed proteins called restriction factors, they can act rapidly to inhibit viral infections at very early stages. A hallmark of all known intrinsic defenses is the presence of a viral countermeasure that inactivates them. When I started my lab, it was known that herpesvirus genomes initiated lytic phase IE transcription near nuclear substructures called PML nuclear bodies (PML-NBs) or ND10s. This led to the hypothesis that PML-NBs enhance viral infections. However, it was well established that herpesviruses and adenoviruses expressed proteins that dissolved or modified PML-NBs, leading to the alternative speculation that they may be detrimental to viral infections. We showed that knockdown of Daxx, a component of PML-NBs, activated HCMV IE transcription, providing the first definitive evidence that PML-NBs were detrimental to viral infections. We described Daxx as mediating an intrinsic cellular defense against HCMV, a nomenclature we adopted from the retrovirus field and which is now in common use by herpes virologists because it accurately and succinctly describes the biological phenotypes instituted by such proteins. Later we showed that BclAF1 also acts in this intrinsic defense, which is actually used by the virus to establish latent reservoirs (see #3 below).

All known intrinsic defenses inhibit productive, lytic replication by inhibiting an essential viral process. But HCMV also establishes latent infections that present a barrier to viral clearance. Latent cells are not cleared by the adaptive immune response, and latent reservoirs are seeded too rapidly for post-exposure prophylactic antiviral treatments to be effective against them. However, our recent work demonstrates that cells latently infected with HCMV are not invisible to the immune system. We discovered that lysine-specific demethylases (KDMs) are restriction factors that prevent HCMV from establishing latency by removing repressive epigenetic modifications from histones associated with the viral Major Immediate Early Promoter (MIEP). KDMs promote transcription of the IE1 gene, whose encoded protein initiates lytic infection and is a prime target of cytotoxic T cells. The viral UL138 protein negates this intrinsic defense by preventing KDM association with the MIEP. Neutralization of this intrinsic defense comes at a fitness cost for HCMV, as UL138 is rapidly lost during serial lytic infections *in vitro*. The presence of an intrinsic defense against latency and the emergence of a cognate neutralizing viral factor indicates that “arms races” between hosts and viruses over lifelong colonization exist at the cellular level. This cell autonomous protection against HCMV latency illustrates that our immune system is geared to deal with viruses that attempt lifelong host colonization by rapidly generating latent reservoirs. This work was facilitated by our recent technical accomplishment of the first ectopic transgene expression during HCMV latency, which required testing multiple promoters inserted into different positions within the viral genome.

a. Lee, S.H., Albright, E.A., Lee, J.H., Jacobs, D.R., and **Kalejta, R.F.** (2015) Cellular Defense Against Latent Colonization Foiled by Human Cytomegalovirus UL138 Protein. Sci. Adv. 1(10)e1501164. PMCID: PMC4681346
b. Qin, Q., Penkert, R.R., and **Kalejta, R.F.** (2013) Heterologous viral promoters incorporated into the human cytomegalovirus genome are silenced during experimental latency. J. Virol. 87, 9886-9894. PMCID: PMC3754107
c. Saffert, R.T., and **Kalejta, R.F., (**2006) Inactivating a cellular intrinsic immune defense mediated by Daxx is the mechanism through which the human cytomegalovirus pp71 protein stimulates viral immediate early gene synthesis. J. Virol. 80 (8), 3863-3871. PMCID: PMC1440479
d. Lee, S.H., **Kalejta, R.F.*,** Kerry, J., Semmes, O.J., O’Connor, C.M., Khan, Z., Garcia, B.A., Shenk, T., and Murphy, E. (2012) BclAF1 restriction factor is neutralized by proteasomal degradation and microRNA repression during human cytomegalovirus infection. Proc. Natl. Acad. Sci. USA, 109, 9575-9580 PMCID: PMC3386064 ***Corresponding Author;**

2. Identification of the v-Cdks

In a landmark paper published in Science and a follow-up manuscript published in PLoS Pathogens, my lab identified the kinase encoded by HCMV, the UL97 protein, as being responsible for Rb protein phosphorylation in HCMV-infected cells. We determined that UL97 and the homologous beta- and gamma-herpesvirus kinases were functional homologs of the cellular cyclin-dependent kinases, thus defining a new v-

Cdk family of kinases. This was the first report of a novel way in which viruses inactivate Rb since the discovery of the KSHV cyclin 13 years prior.

a. Hume, A.J., Finkel, J.S., Kamil, J.P., Coen, D.M., Culbertson, M.R., and **Kalejta, R.F.** (2008) Phosphorylation of retinoblastoma protein by viral protein with cyclin-dependent kinase function. Science, 320, 797-799. PMCID: none
b. Kamil, J.P., Hume, A.J., Jurak, I., Münger, K., **Kalejta, R.F.**, and Coen, D.M. (2009) Human Papillomavirus 16 E7 inactivator of retinoblastoma family proteins complements human cytomegalovirus lacking UL97 protein kinase. Proc. Natl. Acad. Sci. USA 106 (39), 16823-16828. PMCID: PMC2757844
c. Kuny, C.V., Chinchilla, K., Culbertson, M.R., and **Kalejta, R.F.** (2010) Cyclin-dependent kinase-like function is shared by the beta- and gamma- subset of the conserved herpesvirus protein kinases. PLoS Pathog 6(9): e1001092. doi10.1371/journal.ppat.1001092. PMCID: PMC2936540
d. Iwahori, S., Hakki, M., Chou, S., and **Kalejta, R.F.** (2015) Molecular determinants for the inactivation of the retinoblastoma tumor suppressor by the viral cyclin-dependent kinase UL97. J. Biol. Chem. 290, 19666-19680 PMCID: PMC4528131

3. Revealing how the latency / lytic decision is made for HCMV

Daxx-mediated silencing of HCMV IE gene expression (see #1 above) occurs upon viral genome entry into the nucleus of all cell types. This transcriptional repression is counteracted in fully differentiated cells where HCMV initiates a lytic infection when pp71, a component of the virion tegument delivered directly to cells upon viral entry, migrates to the nucleus and degrades Daxx. We showed that in undifferentiated myeloid cells where HCMV establishes latency, tegument-delivered pp71 remains in the cytoplasm, and Daxx silences viral genomes in the nucleus. Knockdown of Daxx (or HDAC inhibition) permits HCMV IE gene expression. Thus, we discovered that HCMV actually uses the cellular protein Daxx to help silence its genome during latency, and that the determining step for the establishment of latency is the sub-cellular localization achieved by tegument-delivered pp71. We find this causal relationship to be true for all cell types tested, where nuclear pp71 initiates lytic replication and cytoplasmic pp71 allows for latency establishment.

a. Albright, E.R., and **Kalejta, R.F.** (2013) Myeloblastic cell lines mimic some but not all aspects of human cytomegalovirus experimental latency defined in primary CD34+ cell populations. J. Virol. 87, 9802-9812. PMCID: PMC3754112
b. Penkert, R.R., and **Kalejta, R.F.** (2013) Human embryonic stem cell lines model experimental human cytomegalovirus latency. mBio 4(3):e00298-13. PMCID: PMC3663570
c. Saffert, R.T., Penkert, R.R., and **Kalejta, R.F.** (2010) Cellular and viral control over the initial events of human cytomegalovirus experimental latency in CD34+ cell. J. Virol. 84 (11) 5594-5604. PMCID: PMC2876595
d. Saffert, R.T., and **Kalejta, R.F.,** (2007) Human cytomegalovirus gene expression is silenced by the Daxx-mediated intrinsic immune defense when model latent infections are established in vitro. J. Virol. 81 (17), 9109-9120. PMCID: PMC1951389

4. Delineating how the ubiquitin – proteasome pathway enhances HCMV infection

We discovered multiple ways in which the ubiquitin – proteasome pathway facilitates HCMV infection. First, cellular proteasomes cooperate with the viral pp71 protein to mediate Daxx degradation, which initiates viral IE gene expression at the start of a lytic infection. Interestingly, this happens in a ubiquitin-independent manner but still requires the 19S regulatory particle, the proteasome adaptor also required for ubiquitin-dependent degradation. The uncommon mechanism of this reaction and its importance to lytic replication makes it a prime drug target. Second, we showed that histone mono-ubiquitination mediated by cellular Elongin B facilitates HCMV IE gene transcriptional elongation. Third, we found the process of transcriptional elongation of HCMV IE genes is also enhanced by the 19S proteasomal regulatory particle in a degradation-independent manner. Our work shows the multiple ways in which the virus corrupts the ubiquitin – proteasome system for its benefit, and the multiple points at which viral IE gene expression is modulated during infection.

a. Winkler, L.L., and **Kalejta, R.F.** (2014) The 19S proteasome activator promotes human cytomegalovirus immediate early gene expression through proteolytic and non-proteolytic mechanisms. J. Virol. 88, 11782-11790 PMCID: PMC4178716
b. Winkler, L.L., Hwang, J., and **Kalejta, R.F.** (2013) Ubiquitin-independent proteasomal degradation of tumor suppressors by human cytomegalovirus pp71 requires the 19S regulatory particle. J. Virol. 87, 4665-4671. PMCID: PMC3624388
c. Hwang, J., Saffert, R.T., and **Kalejta, R.F.** (2011) Elongin B-mediated epigenetic alteration of viral chromatin correlates with efficient human cytomegalovirus gene expression and replication. mBio 2(2):e00023-11.doi:10.1128/mBio.00023-11. PMCID: PMC3063379
d. **Kalejta, R.F.,** and Shenk, T. (2003) Proteasome-dependent, ubiquitin-independent degradation of the Rb family of tumor suppressors by the human cytomegalovirus pp71 protein. Proc. Natl. Acad. Sci. USA 100 (6), 3263-3268. PMCID: PMC152280

5. Surprisingly finding that Rb is required for efficient HCMV replication

DNA tumor viruses have long been known to "inactivate" Rb. Likewise, HCMV encodes at least four proteins (pp71, UL97, IE1, and IE2) that impact the Rb-E2F pathway. I discovered pp71 degrades Rb as a postdoc with Tom Shenk, and my independent lab revealed UL97 phosphorylates Rb (see #2 above). With all the ways HCMV "inactivates" Rb, we were surprised to discover that HCMV replicates less efficiently in Rb knockdown cells. This work was published in two Journal of Virology papers, both picked for the "Spotlight" section of the edition in which they appeared. One of the assumed roles of RB inactivation during DNA virus infection was to stimulate the transcription of the E2F-responsive nucleotide biosynthetic enzymes required for the *de novo* synthesis of these critical precursors of DNA replication. However, we have a manuscript under revision demonstrating that Rb phosphorylation is not required for cells to generate the nucleotides required for HCMV viral DNA synthesis. This helps to highlight how much we don't know about the role of Rb during viral infections. In total our data indicate that HCMV achieves a nuanced modification of Rb function. Understanding the positive impacts that cellular tumor suppressors like Rb have on viral infections may reveal new activities of these well studied yet incompletely understood proteins, and may help in the design of modified oncolytic viruses with greater selective tumor cell replication and killing.

a. VanDeusen, H.R., and **Kalejta, R.F.** (2015) The retinoblastoma tumor suppressor promotes efficient human cytomegalovirus replication. J. Virol. 89, 5012-5021. PMCID: PMC4403481
b. VanDeusen, H.R., and **Kalejta, R.F.** (2015) Deficiencies in cellular processes modulated by the retinoblastoma protein do not account for reduced human cytomegalovirus replication in its absence. J. Virol. In press
c. Kuny, C.V., and **Kalejta, R.F.** "HCMV can procure deoxyribonucleotides for viral DNA replication in the absence of retinoblastoma protein phosphorylation" manuscript under revision

Complete list of published work in My Bibliography (total of 48):

http://www.ncbi.nlm.nih.gov/sites/myncbi/rob.kalejta.1/bibliograpahy/40512254/public/?sort=date&direction=ascending

D. Research Support

Current:

NIH/NIAID 5R01AI074984-07 PI: Kalejta 07/01/07-11/30/18
"Role of Daxx degradation by pp71 during the human cytomegalovirus life cycle"
This grant examines how Daxx and pp71 regulate viral transcription.

NIH/NCI 5P01CA022443-38 PI: Lambert 02/01/97 – 04/30/2018
"Molecular Biology and Genetics of Human Tumor Viruses"

Project 3 Visualizing EBV and HCMV DNA dynamics during infection

Project Leader: Bill Sugden; Co-Leaders: Lambert, PF, and Kalejta, RF

My role is to determine whether or not HCMV replicates its genome during latency.

Project 5 miRNA and v-Cdks: Independent drivers of EBV and HCMV replication and oncogenesis *in vitro* and *in vivo*

Project Leader: Shannon Kenney; Co-Leaders: Sugden, B, and Kalejta, RF

My role is to determine if Hsp90 inhibitors that impair the accumulation of v-CDKs can be used as inhibitors of HCMV replication *in vitro* and in humanized mouse models.

Pending:

None

Recently Completed:

NIH/NIAID 5RO1AI080675-05 PI: Kalejta 05/01/10-04/30/15
"Retinoblastoma (Rb) protein pathway in human cytomegalovirus infected cells"

This grant explores how the Rb pathway regulates HCMV infection. A renewal has not yet been submitted.

Avon Foundation PI: Kalejta 06/01/14-05/31/16
"Human cytomegalovirus breast cancer transcriptome"

This grant uses Agilent Sure Select baits to enrich (>800-fold) for HCMV transcripts present in human tumor biopsy specimens that are then identified and quantitated by deep sequencing.